**Original Research Article** **Bioinformatics**

**International Journal of Pharma and Bio Sciences**

**ISSN 0975-6299**

# MOLECULAR DOCKING AND DYNAMIC SIMULATION OF 3-DEHYDROQUINATE DEHYDRATASE FROM *MYCOBACTERIUM TUBERCULOSIS*

**MUSTAFA ALHAJI ISA, BALWANT KISHAN MALIK**

*Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, Uttar Pradesh, India*

## ABSTRACT

Tuberculosis is the most common cause of death due to an infectious agent known as *Mycobacterium tuberculosis*. Therefore, the aim of this study was to identify novel inhibitors of *3-dehydroquinate dehydratase* of Shikimate pathway using both natural and synthetics ligands libraries. Three dimensional structure (3D) of *3-dehydroquinate dehydratase* was retrieved from Protein Data Bank (PDB ID: 3N76) and subjected to energy minimization and protein optimization. Virtual screening was performed based on physicochemical properties (Lipinski rule of five) and biological activity. Molecular docking analysis of protein-ligand complex was carried out using AutoDock4.0 for both synthetic and natural ligands (PubChem Database and Zinc Database). Molecular Dynamic (MD) simulation was also performed to determine the stability of protein-ligand complex using CHARMM (Chemistry at Harvard Macromolecular Mechanics) force field in NAMD v2.12 (Nanoscale Molecular Dynamic Program). The results of molecular docking revealed that five molecules had high binding energy (ZINC14981770, ZINC01147665, ZINC22910025, ZINC8442077 and PubChem72341) range between -8.99 and -8.39 kcal/Mol. Leu14, Gly15, Gly18, Glu21, Tyr25, Gln52, Asp54, Gln58, Asp76, Gly79, Ile103 and Arg109 stabilised the protein-ligand complex through theformation of hydrogen bonding.Lastly, the ligands were further screening for pharmacokinetic properties based on Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. Therefore, these ligands will be recommended for the treatment of tuberculosis after successful *in vitro* and *in vivo* biological assay.

**KEYWORDS:** ***Nanoscale Molecular Dynamics (NAMD), Molecular Dynamic (MD) Simulation, Mycobacterium tuberculosis, 3- dehydroquinate dehydratase and Docking***

**MUSTAFA ALHAJI ISA**

**Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, Uttar Pradesh, India**

**Received on: 04-08-2017**
**Revised and Accepted on: 21-09-2017**
**DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b314-320**

# INTRODUCTION

Tuberculosis is the most common cause of death due to an infectious agent known as *Mycobacterium tuberculosis* (MTB). It is a devastating disease which leading to higher morbidity and mortality across the world. The present drugs in existence, play animportant role in controlling the disease to the extent that is being done today, although several lacuna attached to it, the most common among them is the emergence of drug resistance making even the frontline drugs inactive. Besides, drugs such as rifampicin have high levels of side effects, devastating to patient incompliance. In addition, most of the antitubercular drugs,lack ability to attack the latent stage of the bacillus. Another major problem is the presence of human immunodeficiency virus (HIV) couple with TB co-infection poses a tremendous challenge for antimycobacterials drug discovery[1]. Despite the presence of vaccine, Bacille Calmette-Guerin (BCG) and short-course chemotherapy, tuberculosis responsible for claiming many lives than any other single infectious agent. In recent years the diseases have seen anincreased incidence especially in developing countries due to widespread of emergence of multidrug-resistant tuberculosis and co-infection with human immunodeficiency virus. Resistance due to multidrug-resistant tuberculosis (MDR-TB) is a serious threat posing a great challenge globally and impede thetuberculosis control programme. Since theadvent of HIV/TB co-infection many people died of tuberculosis[2-5]. Tuberculosis (TB) is the major threat to global health resulting to about 8.6 million people infected in 2012[6]. The World Health Organization (WHO) reported that almost 450, 000 people had multidrug-resistant tuberculosis (MDR-TB) in 2012, in contrast to 62,000 cases in 2011. Of this total, 9.6% cases were found to have extensively drug-resistant tuberculosis (XDR-TB) [6]. Due to rapidly growing MDR-TB and XDR-TB cases, it is necessary for discovery of new and alternative tuberculosis drug that can combat the resistance species of the organism.An alternative and less expensive drugdevelopment is very important to completely eradicate the multidrug resistant cases of the tuberculosis. From 1980 to 2004 about 81 million new cases were reported and based on genome sequencing it clearly indicated that many enzymes can be useful as apotential candidate for computational drug design including *3-dehydroquinate dehydratase* in shikimate pathway from *Mycobacterium tuberculosis*. Shikimate pathway enzymes play indispensable role in the biosynthesis of aromatic amino acids and many other essential compounds in plants, bacteria, fungi and algae, and due to their absences in mammal making them animportant target for development of antimicrobial drugs. It is a biosynthetic pathway that starts with condensation of erythrose 4-phosphate and phosphoenolpyruvate to chorismate which can be achieved in seven steps[7].One of the important enzymes in this pathway is *3-dehydroquinate dehydratase*that catalysing third of the seven steps involved in the pathway. This enzyme responsible for catalysing 3-dehydroquinate to 3-dehydroshikimate by releasing water molecules, thus inhibition of this enzyme is a promising target for thedevelopment of antitubercular drugs. Therefore, the aimed of this study was to determine Insilico docking and molecular dynamic simulation of 3-dehydroquinate dehydratase from *Mycobacterium tuberculosis.*

# METHODOLOGY

### *Protein Optimization and Energy Minimization*

The crystal structure of *3-dehydroquinate dehydratase* was obtained from Protein Data Bank (PDB ID: 3N76) with resolution of 1.9Å and optimized through separating the protein residues and non-protein moieties. To ensure reliable and high quality structure, the protein was subjected to energy minimization with SPDV by adding all missing hydrogens and removal of all water molecules.

### *Binding site prediction*

The active site of the protein was predicted using 3DLigandSite-Ligand binding site prediction Server (http://www.sbg.bio.ic.ac.uk/3dligandsite/)[8], which determined the functional active residues in the target protein that have high capability and potentiality to bind to the ligands.

### *Virtual screening of the ligands*

The compounds with antibacterial activity were obtained from Zinc database and PubChem database public domain through virtual screening using RASPD online tool[9]. The compounds were further filtered for physicochemical properties and drug likeness using Molsoft server based on Lipinski rule of five. Also bioactivity such as GPCR ligand, Ion channel modulator, Kinase inhibitor, nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor of the ligands were determined using Molinspiration online tool and all compounds passed these testswere further used for molecular docking.

### *Molecular docking*

Molecular docking was performed to determine the interaction modes and binding affinities between the modelled structure of *3-dehydroquinate dehydratase* and different ligands using Autodcok4.0[10], which uses a rapid energy evaluation through precalculated grids of affinity potentials with different search algorithms to determine the suitable binding positions for a ligand on a given protein. All torsion angles for each ligand were made to be flexible. The grid maps representing the proteins in the actual docking process were determined using AutoGrid. The dimensions of the grids were set as 60×60× 60 Å, with a spacing of 0.375 Å between the grid points[11].

### *Determination of absorption, distribution, metabolism, excretion and toxicity (ADMET)*

The pharmacokinetic properties of the ligands were determined based on ADMET properties using AdmetSAR online tool (http://lmmd.ecust.edu.cn:8000/predict)[12]. Also mutagenicity of the ligands was determined based on the Ames toxicity test which predicts the tendency of the ligands to cause mutation to human cell**s**. The Admet assay is necessary in drug discovery and development because it's affects the drug levels and exposure of the drug to the tissues and hence affects the performance and pharmacological activity of the ligand as a drug.

### *Molecular Dynamic Simulation*

The molecular dynamic simulation of protein-ligand complex was performed using NAMD-VMD program[13]. The complex was prepared using and chimera[14]and parameterized using SwissParam servers[15]. The coordinate of all atoms in the complex were generated through protein structure file (PSF) and then solvated with a 10.0 Å of TIP3P water box. Finally, the solvated protein-ligand complex were equilibrated and minimized with 1,000 steps and simulation of the complex was performed at 1,000,000 (2ns) runs. The entire trajectory was saved for further analysis.

## RESULTS AND DISCUSSION

### *Protein Optimization and Energy Minimization*

The pandemic effect of multi-drug resistance TB (MR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are global threat. Due to some lacuna attached to standard six month treatment for TB, MDR-TB case become**s** ineffective, costly and time consuming. Therefore, an alternative and less expensive drug development is very important to completely eradicate the multidrug resistant cases of the tuberculosis. Shikimate pathway plays indispensable role in the synthesis of aromatic compounds and many other essential nutrients in plants and microorganisms (bacteria, fungi and algae) and due to their absences in mammal making them animportant target for thedevelopment of antimicrobial drugs. In this study 3-dehydroquinate dehydratase from shikimate pathway of *Mycobacterium tuberculosis* H37Rv was obtained from Protein Data Bank (PDB ID: 3N76) with resolution of 1.9Å. The protein was optimized by removing all non-residues moieties and the energy minimization was carried out by adding all missing hydrogens and removal of all water molecules.

### *Binding site prediction*

The binding site prediction of 3-dehydroquinate dehydratase was performed using 3DLigandSite-Ligand binding site prediction Server, which determines the active site with higher average precision. Leu14, Gly15, Gly18, Glu21, Tyr25, Gln52, Asp54, Gln58, Asp76, Gly79, Ile103 and Arg109 found to be functional active sites of the *3-dehydroquinate dehydratase.*

### *Virtual screening of the ligands and Molecular docking*

The compounds with antibacterial activity were obtained from Zinc database and PubChem database public domain through virtual screening using theRASPD online tool[9]. The total of nineteen thousand seven hundred compounds (19700 compounds) was obtained and further filtered based on physicochemical properties (molecular and drug likeness) and bioactivity. Three hundred and fifty (350) compounds were selected based on the aforementioned rules and subjected to molecular docking to determine the binding poses between the *3-dehydroquinate dehydratase* and ligands using Autodock4.0 (Table 1 and 2). Five compounds showed a promising activity against the *3-dehydroquinate dehydratase* and satisfied Lipinski rule of five and possessed drug likeness properties (Table 3). The biological activities, of the compounds include GPCR ligand also known as 7-Transmembrane receptors (7-TM receptors), which are target of majority of all modern medicinal drugs. They are readily accessible to hydrophilic drugs due to their expression on the cell surface which provides selectivity in activating event. Therefore the antagonist and agonist of 7-TM receptors are essential in thetreatment of disease in every organ system. In this study, three ligands (ZINC14981770, ZINC22910025 and PubChem72341) are antagonists of GPCR ligand while the remaining ligands are agonist. Other biological activities include Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor were determined and all the ligands showed various degree of antagonist and agonist activities (Table 2).The ligands bind to different residues constituting the binding pocket of the protein.The compound with least binding energy was ZINC14981770 with minimum binding affinity of -8.99 kcal/mol and inhibitory constant of 258.55 nM (nanomolar) and formed hydrogen bond withTyr25 and Asn76 residues, followed by ZINC01147665 (minimum binding affinity of -8.57 kcal/mol and inhibitory constant of 525.12 nM (nanomolar ) and formed hydrogen bond withLeu14, Asn76 and Gly78 residues), ZINC22910025 (minimum binding affinity of -8.48 kcal/mol and inhibitory constant of 681.02 nM (nanomolar) formed hydrogen bond withGly11, Gly15, Asp54, His30 and Asp31) and ZINC8442077 (minimum binding affinity of -8.41 kcal/mol and inhibitory constant of 681.02 nM (nanomolar) and formed hydrogen bond withAsp54 and Gly15), on the other hand natural ligand (PubChem72341) had least binding energy of -8.39kcal/mol and inhibitory constant of 712.89 nM (nanomolar) and formed hydrogen bond withGlu21, Asp76, and His102 (Table 3: Figure 1).

**Table 1**

***Physicochemical/molecular and drug likeness properties of ligands with least docking scores***

| S/No. | Zinc /PubChem ID | Molecular weight | Number of HBA | Number of HBD | MolLogP | MolLogS (in Log(moles/L)) | MolPSA ($A^2$) | MolVol ($A^3$) |
|---|---|---|---|---|---|---|---|---|
| | Range value | <=500 | <=10 | <=5 | <5 | | <140 | |
| 1. | ZINC14981770 | 453.27 | 6 | 2 | 1.94 | -1.19 | 67.70 | 466.34 |
| 2. | ZINC01147665 | 472.10 | 6 | 2 | 5.70 | -8.63 | 68.22 | 454.92 |
| 3. | ZINC22910025 | 492.26 | 6 | 0 | 5.42 | -5.24 | 40.24 | 523.84 |
| 4. | ZINC8442077 | 456.10 | 8 | 2 | 1.98 | -5.46 | 99.21 | 448.12 |
| 5. | PubChem72341 | 475.28 | 5 | 3 | 4.11 | -4.87 | 58.36 | 506.73 |

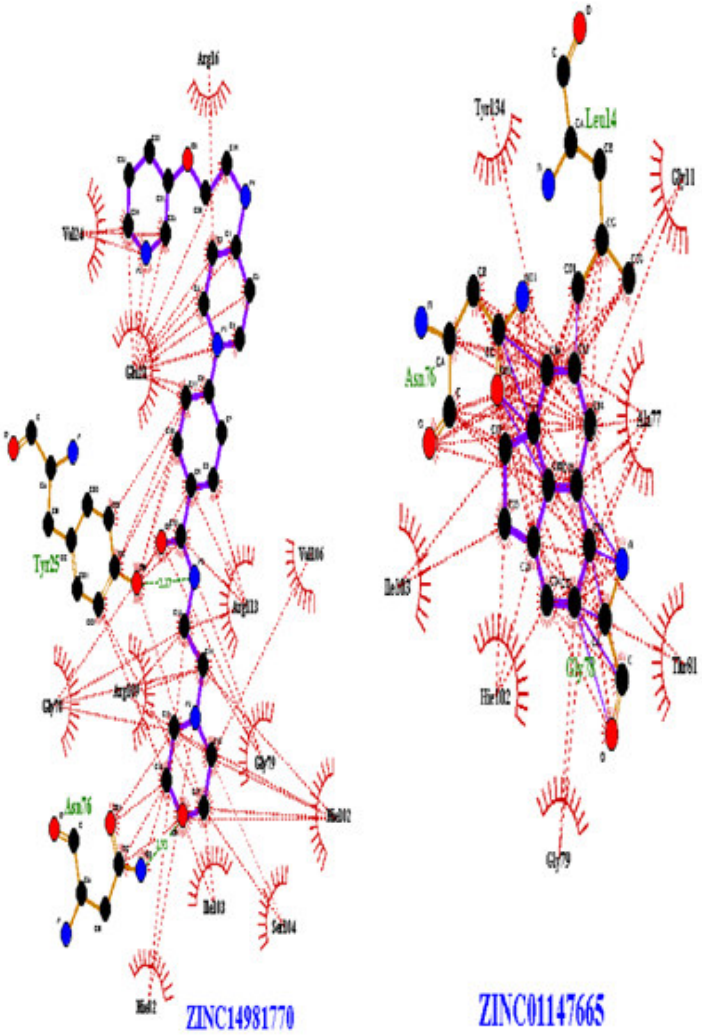
***LogP (octanol/water partition coefficient), LogS (water solubility), Molecular Polar Surface Area (PSA) and Volume***

**Table 2**
***Biological activity of ligands with least docking score***

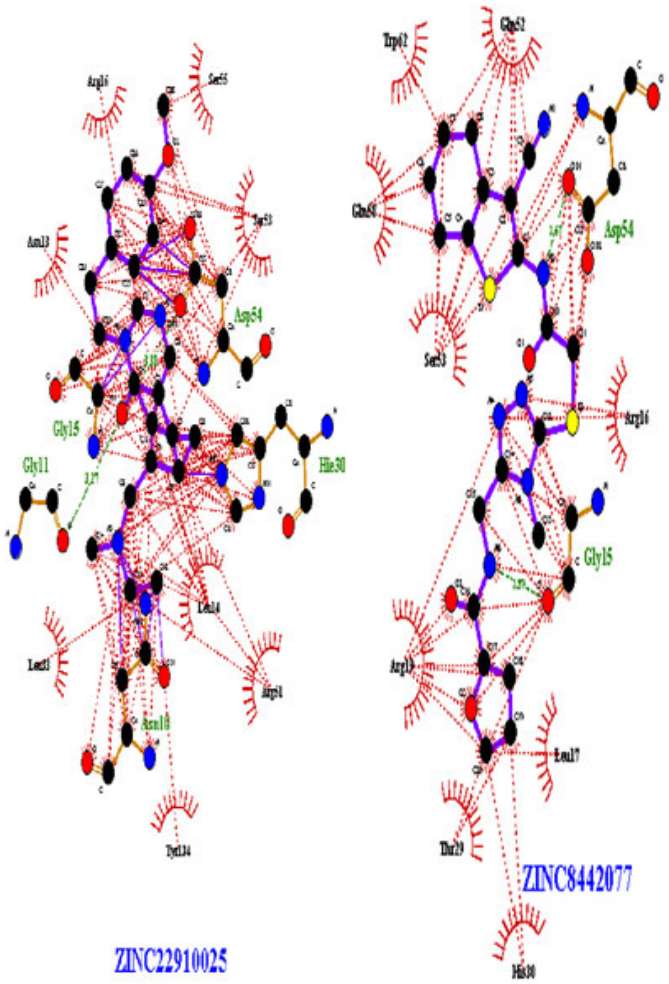
| S/No. | Zinc /PubChem ID | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|---|
| 1. | ZINC14981770 | 0.26 | 0.23 | 0.26 | -0.17 | 0.08 | 0.08 |
| 2. | ZINC01147665 | -0.57 | -0.55 | -0.64 | -0.70 | -0.60 | -0.34 |
| 3. | ZINC22910025 | 0.06 | -0.29 | -0.38 | -1.15 | -0.39 | -0.24 |
| 4. | ZINC8442077 | -0.89 | -1.45 | -0.97 | -1.52 | -1.01 | -0.75 |
| 5. | PubChem72341 | 0.36 | 0.15 | -0.13 | -0.15 | -0.02 | 0.08 |

**Table 3**
***Docking result of 3-dehydroquinate dehydratase with synthetic and natural ligands***

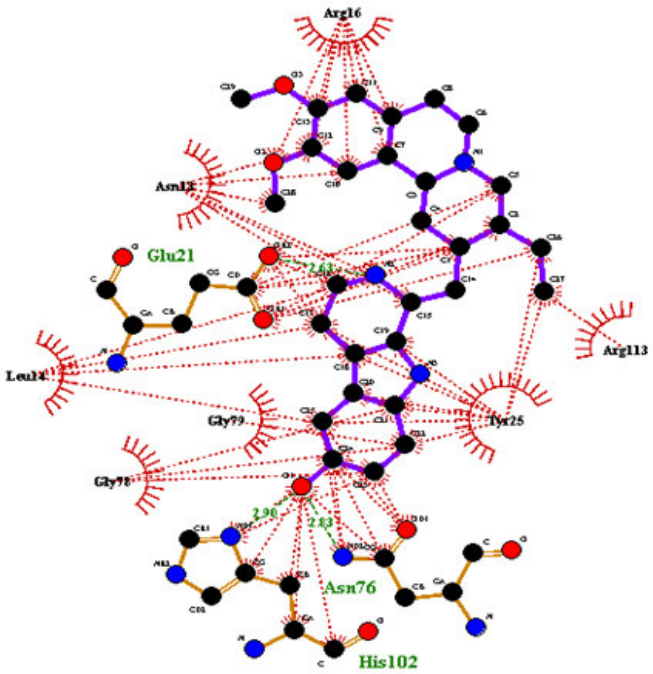
| S/NNo. | Zinc Code | Minimum Free Energy of Binding (kcal/mol) | Inhibition Constant, Ki (nM (nanomolar) | Residues Involved in Hydrogen Bonding | Number Number of Hydrogen Bonding | Residues Involved in hydrophobic interaction |
|---|---|---|---|---|---|---|
| 1. | ZINC14981770 | -8.99 | 258.55 | Tyr25 and Asn76 | 2 | Arg16, Val24, Glu21, Gly78, Arg109, His82, Ile103, Ser104, Gly79, His102, Arg113, Val106 |
| 2. | ZINC01147665 | -8.57 | 525.12 | Leu14, Asn76 and Gly78 | 3 | Tyr134, Ile103, His102, Gly79, Thr81, Ala77, Gly11 |
| 3. | ZINC22910025 | -8.48 | 604.37 | Gly11, Gly15, Asp54, His30 and Asp31 | 5 | Arg16, Asn13, Leu33, Tyr134, Leu14, Arg51, Ser53, Ser55 |
| 4. | ZINC8442077 | -8.41 | 681.02 | Asp54 and Gly15 | 2 | Gln52, Trp62, Gln58, Ser53, Arg19, Thr29, His30, Leu17, Arg16 |
| 5. | PubChem72341 | -8.39 | 712.89 | Glu21, Asp76, and His102 | 3 | Arg16, Asn13, Leu14, Gly78, Gly79, Tyr25, Arg113 |




***(a)*** ***(b)***




*(c)* *(d)*




*(e)*

**Figure 1(a-e)**
***Molecular interaction of best five ligands with functional active site of 3-dehydroquinate dehydratase***

***ADMET properties of ligands***

The ADMET properties of ligands with least docking score were shown in Table 4. The absorption properties of the ligands were tested based on blood brain barrier and human intestinal absorption and all the ligands shown positive to both. Distribution and metabolism were tested based on Cytochrome P450 2D6 (CYP450 2D6) assay and with the exception of ZINC22910025 and PubChem72341, all the remaining ligands were non-inhibitor of the CYP450 2D6 enzymes. Finally, excretion and toxicity assay were determined based on ames toxicity assay, carcinogens assay and biodegradation assay and clearly unravel all the ligands were neither toxic nor carcinogens to human cell (Table 4). The combination of molecular docking, virtual screening and molecular dynamic simulation of 3-dehydroquinate dehydratase performed in this study can be further evaluated by both in vivo and in vitro biological assay.

***Molecular Dynamic Simulation***

Molecular dynamic simulation was performed to determine the stability or dynamic properties of protein-ligand complex. The result shows that the overall conformation of the complex. The overall system was monitored using root mean square deviation (RMSD) with Phi and Psi value. The protein-ligand complex was simulated for 2000 ps and flexibles amino acids residues were found in the active site which gives a reliable conformationof the complex, due to hydrogen bonding and hydrophobic interaction (Figure 2). Thus, the trajectories analysis shows that ligand was stable within the binding pocket of the 3-dehydroquinate dehydratase (Glu21, Tyr25, Asn76 and Ile103).

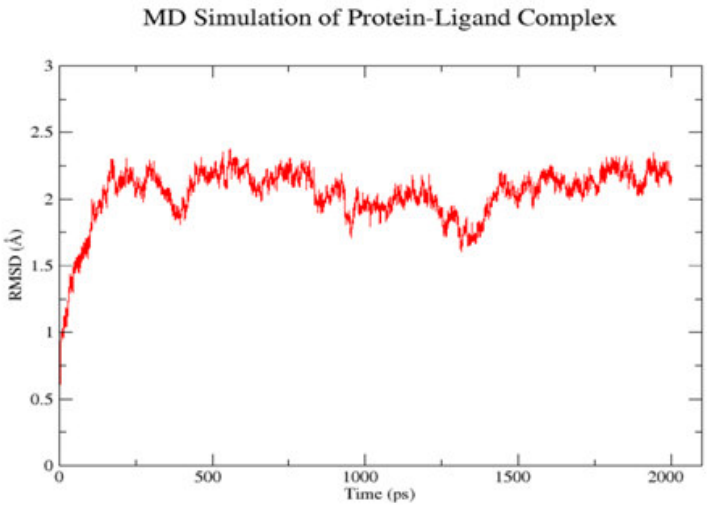



**Figure2**

***Root mean square deviation of 3-dehydroquinate dehydratase- ZINC14981770 complex runs for 2ns***

**Table 4**

***ADMET properties of ligands with least docking score***

| S/NO. | Zinc /PubChem ID | Absorption | | | | Distribution and Metabolism | | Excretion and Toxicity | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Blood-Brain Barrier | Result | Human Intestinal Absorption | Result | CYP450 2D6 Inhibitor | Result | AMES Toxicity | Result | Carcinogens | Result | Biodegradation | Result |
| 1. | ZINC14981770 | 0.9366 | BBB+ | 0.9360 | HIA+ | 0.7684 | Non-Inhibitor | 0.6117 | Non Ames toxic | 0.8696 | Non-carcinogens | 0.9722 | Not ready biodegradable |
| 2. | ZINC01147665 | 0.9772 | BBB+ | 0.9853 | HIA+ | 0.8207 | Non-Inhibitor | 0.6160 | Non Ames toxic | 0.8117 | Non-carcinogens | 0.9920 | Not ready biodegradable |
| 3. | ZINC22910025 | 0.8704 | BBB+ | 0.9412 | HIA+ | 0.6153 | Inhibitor | 0.5856 | Non Ames toxic | 0.9202 | Non-carcinogens | 0.5086 | Not ready biodegradable |
| 4. | ZINC8442077 | 0.9134 | BBB+ | 0.9746 | HIA+ | 0.7973 | Non-Inhibitor | 0.5759 | Non Ames toxic | 0.7555 | Non-carcinogens | 0.9870 | ready biodegradable |
| 5. | PubChem72341 | 0.5350 | BBB+ | 0.9754 | HIA+ | 0.9025 | Inhibitor | 0.7994 | Non Ames toxic | 0.9188 | Non-carcinogens | 1.0000 | Not ready biodegradable |

***Blood-Brain Barrier: BBB+ (positive) and BBB- (negative), Human Intestinal Absorption: HIA+ (positive), HIA-(negative)***

## CONCLUSION

*3-dehydroquinate dehydratase* is an important enzyme in shikimate pathway which was considered as a best target for drug development against *Mycobacterium tuberculosis*.. Leu14, Gly15, Gly18, Glu21, Tyr25, Gln52, Asp54, Gln58, Asp76, Gly79, Ile103 and Arg109 found to be a functional active site of the model protein. Five compounds indicated high activity and great potential to use as anti-tubercular drugs (possessed minimum binding energy/docking score, fulfilled Lipinski rule of five and passed ADMET test). Therefore, these natural and synthetic ligands will be used for the treatment of tuberculosis after successful pass *in vitro* and *in vivo* evaluations of bioactivity.

## ACKNOWLEDGMENT

The corresponding author of this paper is very much thankful to Professor N. B. Singh of Department of Research and Development, Sharda University for his tremendous support and encouragement during the course of the research.

## CONFLICT INTEREST

Conflict of interest declared none.

## REFERENCES

1. Reichau S, Jiao W, Walker SR, Hutton RD, Baker EN, Parker EJ. Potent Inhibitors of a Shikimate Pathway Enzyme from Mycobacterium tuberculosis combining mechanism-And modeling-based design. Journal of Biological Chemistry. 2011 May 6;286(18):16197-207.
2. Jothieswari D, Bhaskar Reddy K.Molecular Docking studies of potential chemical inhibitors on multi-drug resistance genes in *Mycobacterium tuberculosis*.I J Innov Drug Discov. 2015;5(1): 40-45.
3. Bello HS, Isa MA, Bintube M, Hassan Z. Prevalence of pulmonary tuberculosis among patient attending some selected hospitals at Maiduguri Metropolis, Borno State, Nigeria.Basic Res J Med Clin Sci. 2014 Dec 4; 3(12): 127-130.
4. Muhammad KU, Bello M, Manga BS, Isa MA. Comparison between Bleach Concentration Method and Conventional Ziehl Neelsen Technique of Determination of Mycobacterium Tuberculosis in Sokoto Metropolis. International Journal of Research. 2014 Dec 1;1(11):504-11.
5. Isa MA,Malik BK.Insilico Identification of Novel Inhibitors for Shikimate Kinase from *Mycobacterium Tuberculosis* using Natural and Synthetic Ligands. J. Chem. Pharm. Res.2016 Dec 16;8(10):25-34.
6. Gordon S, Simithy J, Goodwin DC, Calderón AI. Selective Mycobacterium tuberculosis shikimate kinase inhibitors as potential antibacterials. Perspectives in medicinal chemistry. 2015;7:9.
7. Sinha S, Rajasulochana P, Ramesh Babu PB, Krishnamoorthy P. Comparative modeling of shikimate kinase (M Tb) and molecular docking studies of its known inhibitors. Res J Pharm Biol Chem Sci. 2013;4:715-20.
8. Wass MN, Kelley LA, Sternberg MJ. 3DLigandSite: predicting ligand-binding sites using similar structures. Nucleic acids research. 2010 May 29; 38(suppl2):W469-73.
9. Mukherjee G, Jayaram B. A rapid identification of hit molecules for target proteins via physico-chemical descriptors. Physical Chemistry Chemical Physics. 2013;15(23):9107-16.
10. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of computational chemistry. 1998 Nov 15;19(14):1639-62.
11. La Motta C, Sartini S, Mugnaini L, Simorini F, Taliani S, Salerno S, Marini AM, Da Settimo F, Lavecchia A, Novellino E, Cantore M. Pyrido [1, 2-a] pyrimidin-4-one derivatives as a novel class of selective aldose reductase inhibitors exhibiting antioxidant activity. Journal of Medicinal Chemistry. 2007 Oct 4;50(20):4917-27.
12. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, Lee PW, Tang Y. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. 2012; 3099-3105
13. Nelson MT, Humphrey W, Gursoy A, Dalke A, Kalé LV, Skeel RD, Schulten K. NAMD: a parallel, object-oriented molecular dynamics program. The International Journal of Supercomputer Applications and High Performance Computing. 1996 Dec;10(4):251-68.
14. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera—a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004 Oct 1;25(13):1605-12.
15. Zoete V, Cuendet MA, Grosdidier A, Michielin O. SwissParam: a fast force field generation tool for small organic molecules. Journal of computational chemistry. 2011 Aug 1;32(11):2359-68.

## Reviewers of this article

---

**Vasudev Pai M Pharm (PhD)**

Assistant professor
Department of Pharmacognosy
Manipal College of Pharmaceutical Sciences Manipal University

**Asst. Prof. Dr. Deepansh Sharma, M.Sc, M.Phil, Ph.D.**

Assistant Professor, School of Biotechnology and Bioscience, Lovely Professional University, Phagwara, Punjab, India

**Prof.Dr.K.Suriaprabha**

Asst. Editor , International Journal of Pharma and Bio sciences.

**Prof.P.Muthuprasanna**

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript